Turun yliopisto
University of Turku

BUMBLEBEES IN A CHANGING CLIMATE:

Evaluating the Effects of Temperature on Queen Performance

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TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. A II osa - tom. 301 | Biologica - Geographica - Geologica | Turku 2015

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ISBN 978-951-29-6091-0 (PRINT)
ISBN 978-951-29-6092-7 (PDF)
ISSN 0082-6979
Painosalama Oy - Turku, Finland 2015

"*You can, you should,*
and if you're brave enough to start, you will."

— Stephen King, On Writing:
A Memoir of the Craft"

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ABSTRACT

Bumblebees in a Changing Climate: Evaluating the Effects of Temperature on Queen Performance

Bumblebees are a very essential group of pollinating insects, but their populations have declined drastically during the past decades. We need to understand why their numbers are decreasing and what can be done to reverse this trend. Climate change-related phenomena, such as changes in the overwintering temperatures and spring conditions, are among the most prominent threats to bumblebees.

Queens have a special role in the lifecycle of bumblebees because they overwinter and start new colonies the next year. Their successful performance: survival, overwintering ability, longevity, immune competence, and nest establishing capability in spring, is highly important for bumblebee populations. However, the effects of climate change on bumblebee queen performance remain unknown. The main objective of this thesis was to assess how temperature affects the performance of bumblebee queens during and after overwintering.

The effects of warm temperature predicted by climate change scenarios on queen survival and stress-tolerance were studied by a four-month artificial diapause of bumblebee queens at two temperatures (9°C and 1.8°C). Bumblebee colonies were also reared in a laboratory and factors affecting colony characteristics were examined. In addition, queen performance during spring was studied in a starvation experiment using two temperatures (15°C as normal; 24°C as warmer than average) and queens collected from nature right after their emergence.

My research revealed how temperature affects queen performance, and queen size was found to be an important factor determining the direction of some of these effects. We found a 0.4g weight threshold for bumblebee queens to be able to survive overwintering. In addition, during mild winters, larger queens have a higher chance than smaller ones to survive through winter and also to cope with immunological stresses after overwintering. During cold conditions, which are normal in the current climatic situation, this advantage disappears. In the spring starvation experiment, the starved queens survived approximately eight days longer in 15°C than in 24°C, which means that starvation risk rises significantly with increasing spring temperature, in a situation where food is scarce due to for example frost damage or asynchrony between bumblebees and their important food plants.

These results could mean that in the future climate, larger queens are better able to survive the winter, initiate their nests and start rearing their offspring. This may be problematic, because I also detected two alternative strategies of colony development that differ between large and small queens; larger queens start to lay eggs earlier at nest initiation, their colonies mature later, they produce more workers, and they have a more strongly male biased sex allocation compared with smaller queens. If larger queens have a greater change of producing offspring after a mild winter, this could lead to a significant decline in the total production of new queens at a population level. Thus, it seems that queen size could act as one mechanism regulating the population level outcomes in different temperatures.

The new information presented in my thesis reinforces that basic research, monitoring, and local species conservation of bumblebees both in Finland and globally must be increased to ensure that this highly important pollinator group survives in the face of climate change.

TIIVISTELMÄ

Kimalaiset muuttuvassa ilmastossa: lämpötilan vaikutukset kuningatarten suoriutumiskykyyn

Kimalaiset ovat erittäin tärkeä pölyttäjäryhmä, mutta niiden kannat ovat laskeneet rajusti viime vuosikymmenien aikana. Meidän pitäisi selvittää mistä väheneminen johtuu ja miten suuntaus voitaisiin kääntää. Ilmastonmuutoksen välillisesti aiheuttamat ilmiöt, kuten muutokset talvehtimislämpötilassa ja keväisissä olosuhteissa, kuuluvat suurimpiin kimalaispopulaatioita uhkaaviin riskitekijöihin.

Kuningattarilla on tärkeä rooli kimalaisten elinkierrossa, koska ne talvehtivat ja aloittavat uudet yhteiskunnat seuraavana vuonna. Niiden hyvä suoriutumiskyky: elossa säilyvyys, talvehtimiskyky, pitkäikäisyys, immuunipuolustuksen vahvuus ja valmiudet perustaa pesä keväällä, on erittäin tärkeää kimalaispopulaatioille. Ilmastonmuutoksen vaikutuksia kuningatarten suoriutumiskykyyn ei kuitenkaan vielä tiedetä. Väitökseni päätavoitteena oli tutkia ilmastonmuutokseen liittyvien tekijöiden, kuten talvehtimislämpötilan ja keväisten olosuhteiden vaikutuksia kimalaiskuningatarten suoriutumiskykyyn talvehtimisen aikana ja sen jälkeen.

Ilmastonmuutosmallien ennustamien lämpötilojen vaikutuksia kuningatarten stressinsietokykyyn ja selviytymiseen tutkittiin neljä kuukautta kestäneessä kokeessa, jossa kuningattaret talvehtivat kahdessa eri lämpötilassa (9°C ja 1,8°C). Tämän lisäksi kimalaisyhteiskuntia kasvatettiin myös laboratoriossa ja yhteiskuntarakenteeseen vaikuttavia tekijöitä tutkittiin. Kuningatarten keväistä suoriutumiskykyä sen sijaan tutkittiin näännytyskokeessa, jossa luonnosta heti heräämisen jälkeen kerättyjä kuningattaria pidettiin kahdessa lämpötilassa (15°C / normaali; 24°C / tavallista lämpimämpi).

Tutkimukseni paljasti, miten lämpötila vaikuttaa kuningatarten suoriutumiskykyyn, ja kuningattaren koon havaittiin olevan tärkeä tekijä joidenkin vaikutusten suunnan määrittäjänä. Löysimme 0,4 g painorajan, jota vähemmän painavat kuningattaret eivät selvinneet hengissä talvehtimisesta. Lisäksi, tavallista lämpimämpien talvien aikana suuremmilla kuningattarilla näyttäisi olevan paremmat mahdollisuudet kuin pienillä selviytyä talven yli ja myös paremmat valmiudet käyttää immuunipuolustustaan stressitekijöiden torjunnassa. Kylmissä talvehtimisolosuhteissa, jollaiset ovat tyypillisiä tämän hetkisessä ilmastossamme, tämä etu kuitenkin häviää. Keväisessä näännytyskokeessa ilman ravintoa olleet kuningattaret selviytyivät hengissä noin kahdeksan päivää kauemmin 15°C :n kuin 24°C :n lämpötilassa, mikä tarkoittaa, että nälkiintymisriski nousee merkittävästi keväisten lämpötilojen noustessa, jos ravintoa ei ole saatavilla kasvien asynkronian tai pakkasvaurioiden takia.

Tulokset voivat tarkoittaa, että tulevissa ilmasto-oloissa suuremmilla kuningattarilla on paremmat mahdollisuudet selviytyä talvehtimisesta, aloittaa pesät, ja kasvattaa jälkikasvu. Tämä voi olla ongelmallista, sillä havaitsin myös kaksi yhteiskuntarakenteen kehitykseen vaikuttavaa strategiaa, jotka poikkeavat suurten ja pienten kuningatarten välillä: suuremmat kuningattaret aloittavat muninnan aikaisemmin, niiden yhteiskunnat saavuttavat sukukypsän vaiheen aikaisemmin, ne tuottavat enemmän työläisiä ja niillä on pienempiä kuningattaria koirasvoittoisempi sukupuolijakauma. Jos suurilla kuningattarilla on paremmat mahdollisuudet jälkeläistuotantoon lämpimän talven jälkeen, tuotettujen uusien kuningattarien kokonaismäärä populaatiossa saattaa laskea merkittävästi. Kuningattaren koko näyttäisi siis toimivan yhtenä mekanismina säätelemässä populaatiotason vaikutuksia eri lämpötiloissa.

Väitöskirjassani esitetty uusi tieto painottaa, että kimalaisten ja muiden mesipistiäisten perustutkimusta, seurantaa, sekä paikallisten lajien suojelua tulisi lisätä sekä Suomessa että maailmanlaajuisesti, jotta varmistaisimme, että tämä tärkeä pölyttäjäryhmä selviytyy ilmastonmuutoksen aiheuttamista ongelmista.

LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and one manuscript, which are referred to in the text by their Roman numerals:

I **Vesterlund, S.-R.**, Sorvari, J., & Vasemägi, A. (2014) Molecular identification of cryptic bumblebee species from degraded samples using PCR-RFLP approach. *Molecular Ecology Resources* 14: 122–126. doi: 10.1111/1755-0998.12168

II **Vesterlund, S.-R**., Kakko, M., Vasemägi, A., & Sorvari, J. (2014) Status and monitoring of the buff-tailed bumblebee *Bombus terrestris* in Southern Finland. *Entomologica Fennica* 25: 49–56.

III **Vesterlund, S.-R.**, Lilley, T. M., Van Ooik, T., & Sorvari, J. (2014) The effect of overwintering temperature on the body energy reserves and phenoloxidase activity of bumblebee *Bombus lucorum* queens. *Insectes Sociaux* 61: 265–272. doi: 10.1007/s00040-014-0351-9

IV **Vesterlund, S.-R**. & Sorvari, J. (manuscript) The impact of queen size and colony characteristics on sex ratio variation in the bumblebee *Bombus lucorum*.

V **Vesterlund, S.-R**. & Sorvari, J. (2014) Longevity of starved bumblebee queens (Hymenoptera: Apidae) is shorter at high than low temperatures. *European Journal of Entomology* 111(2): 217–220. doi:10.14411/eje.2014.035.

1. INTRODUCTION

1.1. The Decline of Bees

Insects have existed for more than 400 million years and they have a very rich evolutionary history, which has resulted in the intricate web of interactions between other insects, plants, and the rest of the ecosystem. Insects are the richest group of the ~1.2 million (Mora et al. 2011) scientifically valid animal and plant species described until today, with almost 60% of the species (Stork 1988, Samways 1994). Of the estimated total of ~8.7 million species on Earth (Mora et al. 2011), insects would make an appreciable 5.2 million. The prime importance of insects as a group is moving energy through food webs by for example converting plant biomass to animal energy, decomposing organic substances, and simply by being part of the food chain as valuable prey. Some insect species are serious pests, and some act as human parasites or vectors for diseases. On the other hand, some insects work as ecosystem engineers (Jones et al. 1994) that have both direct and indirect effects to the availability of resources to other species. For example, social insects, such as termites and ants, whose colony sizes can reach 12 million even in the temperate areas (Wilson 1971), effectively modify their surroundings and create new habitats.

For humans, insects offer many ecosystem services, such as resources for biological control. One of the most important ecosystem service, however, is the pollination of both wild flowering plants and various crop species: 22 billion Euros are contributed yearly by bees and other pollinators to European agriculture alone (The IUCN Red List of Threatened Species 2014). This is why the recent worldwide decline of bee populations (for example, Williams & Osborne 2009, Ratnieks & Carreck 2010) is raising a lot of concern. According to a recent report in The IUCN Red List of Threatened Species (IUCN 2014), twenty-four percent of the European bumblebee species are threatened by extinction. The negative trends in European bumblebee populations, where almost half of the bumblebee species are declining (The IUCN Red List of Threatened Species 2014), will have to be reversed by protecting bumblebee species and habitats, restoring degraded ecosystems, and promoting agricultural practices that are more biodiversity-friendly. The strategy to halt biodiversity loss in Europe is under review (European Parliament Resolution of 20 April 2012). This stresses the need for conservation efforts for the EU 2020 Biodiversity Strategy to be fully implemented, and in order to fulfill the 2020 biodiversity target in halting biodiversity loss and the degradation of ecosystems services. There are several known causes for the decreased bee numbers. I will next

shortly review the most important factors contributing to bumblebee and bee population declines:

1.1.1 Habitat Loss and Fragmentation

By the end of the twentieth century, farming practices in Europe changed considerably in order to enhance yields and overall productivity in agriculture. New practices were based on monocultures, ploughing old pastures, and the use of fertilizers, which all combined resulted in the loss of important habitats for bees, especially hedgerows and unimproved grasslands (Howard et al. 2003, Goulson 2010). In a Finnish study of the responses of pollinators to vegetation succession in a long-term set-aside, bumblebee numbers peaked rapidly when floral resources were increased (Alanen et al. 2011) indicating the importance of a diverse vegetation in maintaining healthy bumblebee populations. The decline in bee diversity in agricultural areas is mainly caused by decreases in the species abundance and diversity of flowering plants (Banaszac 1992, Gathmann et al. 1994, O'Toole 1994). Today, the number of available food plants for bees is further reduced, because selective herbicides are used to very effectively eliminate broad-leaved weeds among and near the crops (Haughton et al. 2003, Hawes et al. 2003). Modern farming practices have also reduced the number of suitable nesting sites for both below and above ground nesting species by either destroying the sites (e.g., Banaszac 1983) or lowering the number of mammal species such as voles, whose old cavities are often reused especially by bumblebees (Goulson 2010).

1.1.2 Pesticides

The impact of pesticides to the decline of bees has been under intense discussion during the past few years, and there is a growing body of evidence showing negative effects for various pollinator groups. Organic insecticides became known after the Second World War, during which the compounds were developed. Presently, among the most widely used insecticides are neonicotinoids and fipronil, which are highly toxic to invertebrates (Bonmatin et al. 2014). Bees and other pollinators can be exposed to these compounds directly through the spray or dust, pollen, nectar, and guttation drops (Bonmatin et al. 2014). In addition to direct effects, pesticides may cause indirect, sub-lethal effects by, for example, affecting colony development: bumblebee colonies treated with neonicotinoids in an experimental study suffered an 85 % reduction of new queens (Whitehorn et al. 2012). Pesticide risk assessments for honeybees are conducted as a routine, but even though most results can be applied to closely related groups, there are indications that the responses may differ significantly between different bee species or developmental stages. For example, a neurotoxin

called spinosad has been deemed harmless to adult honeybees, but when worker bees were exposed to this pesticide as larvae, their foraging ability as adult workers was reduced (Morandin et al. 2005). The safe concentrations may also vary among different pollinator groups. In a study where eight commonly used pesticides were screened for their negative effects, severe sub-lethal effects to bumblebees were found for diflubenzuron and teflubenzuron even at concentrations significantly lower than those recommended for field use, even though these compounds were not lethal to adult bumblebees (Mommaerts et al. 2006). In response to the risk assessment done by the European Food Safety Authority (EFSA), which identified the risks of these insecticides, the European Union voted for a ban on neonicotinoids in 2013. Recently, pesticides have even been linked to the Colony Collapse Disorder (CCD discussed later in more detail) of honeybees (Henry et al. 2012).

1.1.3 Non-native bees

Honeybees (*Apis mellifera*) have been introduced to almost every country in the world. They are highly polylectic and can be used to pollinate a vast selection of plants. Honeybees are strong competitors and they have even displaced native organisms by disturbing and suppressing their resource availability (Goulson 2003). Especially in northern Europe, honeybees most likely could not sustain high numbers or even persist over winters in native colonies. Thus, commercial beekeeping has the potential to negatively affect other insects using the same floral resources (Goulson 2004). As an example, short-tongued bumblebee species avoid foraging near honeybee hives, and carder bumblebees (subgenus *Thoracobombus*) change into less-preferred food plants and modify their foraging times when honeybees are present (Walther-Hellwig et al. 2006). Also, bumblebee foragers of at least four common species were found to be smaller than average when honeybees were present (Goulson & Sparrow 2009). This would suggest either that because of competition by honeybees, bumblebees could not feed their larvae well enough to produce larger workers, or that the smaller workers usually maintaining the nest were forced to forage due to food scarcity. In experimental studies introducing honeybees outside their native range, bumblebee foraging rates and reproductive success have been reduced in the proximity of honeybee hives (Thomson 2004). In addition to competition, honeybees can act as vectors for parasites and viruses that are then spread to natural bee and bumblebee populations. Ruiz-Conzàlez and Brown (2006) found that honeybees carry bumblebee specific parasites (*Crithidia bombi*) and transport them into the flowers bumblebees use as forage. Another example of honeybee transmitted pathogens is the deformed wing virus, which spread first into commercial bumblebees because honeybee workers were used to induce the colony founding of bumblebee queens (Genersch et al. 2006).

Some species have a greater ability to disperse and conquer new habitats than others. If they are misplaced unintentionally, or moved from their native range on purpose, they often take over and thrive in new environments. These species are called invasive and are defined as being able to disrupt or significantly modify the ecosystem it colonizes. Usually the term "invasive" is particularly used when discussing alien species, i.e., species foreign to the environment in question. The term introduced species, on the other hand, is used when referring to an alien species that has deliberately been presented into an area where it would not naturally occur. The introduction of commercial *B. terrestris* strains to the ecosystem is causing multiple problems around the world. In the countries within the southern hemisphere (New Zealand, Australia including the island of Tasmania), where there originally were no bumblebees, the introduced *B. terrestris* has become naturalised in the wild (Semmens et al. 1993, Hingston & McQuillan 1998, 1999, Hingston et al. 2002, Hergstrom et al. 2002, Goulson & Hanley 2004). In countries such as Japan and Chile, which have their own unique native bumblebee fauna, *B. terrestris* has become the dominant bumblebee species in some areas after its introduction (Matsumura et al. 2004). Interspecific mating can occur in areas where the males of commercial *B. terrestris* are in close contact with local, non-sympatric species of the same subgenus (Kanbe et al. 2008).

In Japan, *B. terrestris* has shown decreased pollination efficiency in native plants, which has caused disturbances in the natural pollination system (Kenta et al. 2007). In Australia, *B. terrestris* has competed with other native pollinators (Hingston & McQuillan 1998), and in Japan, also with native bumblebees (Inoue & Yokoyama 2006). Its commercial strains with large workers have the ability to outcompete and replace native populations through their superior foraging efficiency (Ings et al. 2005). In addition, non-native parasites are spreading into native bumblebee populations from commercial nests (Goka et al. 2001). The prevalence of common parasites (mainly *Apicystis bombi* and *C. bombi*), and the species richness of all studied parasites were found to be higher around greenhouses that use commercial pollinators than further away (Graystock et al. 2014). This would suggest that parasites are spreading from imported colonies, or that the native bumblebees are more easily infected because of the increased stress caused by competing with non-native pollinators. Also, 77% of tested commercially produced bumblebee colonies that were imported to UK in 2011-12 were found to carry microbial parasites that were infectious to bumblebees and some even to honeybees (Graystock et al. 2013). The rapid decline of very common bumblebee species raises serious concerns about the negative effects of introduced pathogens such as the microsporidian gut parasite *Nosema bombi*, which was possibly spread by imported alien bees used for the

pollination of flowering crops (Thorp 2005, Goulson et al. 2008, Cameron et al. 2011, Murray et al. 2013).

1.1.4 Colony Collapse Disorder

The sudden disappearance of honeybees from their hives, later called the Colony Collapse Disorder (CCD), was first identified in 2006 (Watanabe 2006). Since then, the causes for bee losses have been intensively studied and speculated, but mechanisms of CCD have remained elusive. One, at the time a newly identified pathogen, the Israeli acute paralysis virus (IAPV), was concluded as a marker for CCD, but it could not be confirmed as the cause of bee disappearances (Cox-Foster et al. 2007). It has been speculated that some mites such as the ectoparasite *Varroa destructor* have to be present in order for a particular pathogen to be able to kill the whole colony of bees, but that mite interactions cannot explain all colony losses attributed to CCD (Ratnieks & Carreck 2010). Also the obligatory bumblebee associate *Parasitellus fucorum* (Rozej et al. 2012) has the potential of being harmful if the numbers present on an individual bee are high enough to prevent normal movement, or the host is in poor condition because of other stresses (Chmielewski & Baker 2008). In 2012, Henry et al. published an experimental study showing that non-lethal amounts of a common pesticide thiamethoxam affected the colony enough to cause its collapse with the used neonicotinoid causing the honeybee workers to lose orientation while foraging. Homing failure led to decreased worker survival especially in sites where homing task was rated challenging (Henry et al. 2012). The most prevailing hypothesis today seems to be that many agents in combination are causing bee colonies to perish, for instance the interactions between parasites and pathogens, food scarcity, pesticide effects, poor weather conditions during critical times in the colony cycle, stress caused by long-distance transportation of the hives, just to mention a few possibilities.

1.1.5 Climate Change

Climate change induced phenomena are among the main threats to bee species. Climate change due to increased atmospheric concentrations of greenhouse gases is often defined as a significant difference in average weather conditions lasting for an extended period of time. There are several models that are used to predict future climatic conditions on Earth, and important modes of seasonal phenomena and climate variability can be generated by modeling (Flato et al. 2013). The confidence levels of these models are improving all the time, and they can simulate with very high accuracy, for example, ocean heat uptake, variations caused by volcanic eruptions, and earth surface temperature, which all reproduce the general features of global mean temperature increase over historical periods of time (Flato et al. 2013).

Worst case scenario models produced by the Intergovernmental Panel on Climate Change (IPCC) predict that the mean global temperature may rise as much as 4.8°C during the next hundred years, affecting especially winter temperatures in Fennoscandia (IPCC 2013). According to one of the projections obtained using high resolution observational data, the thermal winter is going to disappear in the South-western part of Finland, and in other parts of the country, the winter will be on average two to four months shorter by the beginning of the next millennium (Ruosteenoja et al. 2014). This is also consistent with climate models derived from month-, location-, and model-specific threshold temperatures of a simulated 20th century climate, which predict that the cold months are becoming less common (Räisänen & Ylhäisi 2011). Further, decreases in near surface permafrost and northern high-latitude spring time snow cover are expected as global mean surface temperature rises (Kirtman et al. 2013). The examination of geographical patterns shows also that snow cover is most vulnerable in areas with relatively mild winter climate (Räisänen & Eklund 2012).

Climate change shifts the natural balance of many species (Thomas et al. 2006). Together with the effects of habitat degradation, climate change has already triggered extinctions, for example, of several frog species through fungal epidemics (Pounds et al. 2006). The distribution of some butterfly species has shifted to higher elevations due to climate-mediated habitat loss at warm margins of their range (Wilson et al. 2005). Viewed from the bumblebee's perspective, climate change may affect the seasonal availability of important flowers such as willows in the spring, if they advance phenology (Forrest & Thomson 2011) or are damaged by extreme weather conditions such as frost (Inouye 2008). Overwintering insects with a tight energy budget are especially vulnerable to increased temperatures (Hahn & Denlinger 2011) and warm winters are likely to increase energy expenditure during winter diapause. Warm winters may lengthen the life of the colony and create second generations. In Britain, the buff-tailed bumblebee, *B. terrestris*, can stay active during winter months, which indicates that this species is capable of maintaining its foraging rates even through abnormal seasons (Stelzer et al. 2010).

In addition to a long term increase in surface temperature, also drought and heat waves may affect the bumblebees and lead to very strong local reductions of the bumblebee fauna. Heat waves occur when the daily maximum temperature exceeds the average maximum temperature by 5°C (World Meteorological Organization 2014). A sudden increase in short-time mean temperatures combined with the decrease of yearly precipitation have caused several occasions of the Bumblebee Scarcity Syndrome (BSS). For example, in Finnish Lapland (Utsjoki), there was a record temperature of 33°C in July 2003, after which the number of observed bumblebees declined severely (Rasmont & Iserbyt 2012). Drought and excess heat are expected to affect all bumblebee species. However, species

with a late summer phenology, such as *B. magnus* in the subgenus *Bombus* (*s. str.*), are the most sensitive to BSS related threats because, at the most common time of heat waves in July, their colonies are still active and producing the sexual progeny (Robinson 2001). The outcome at the population level will depend both on the continuity of these heat wave events (Rasmont & Iserbyt 2012) and the duration of each individual event (Denlinger & Yocum 1998).

In Finland, the climate change predicted using observational data would lengthen the summer by over one month, and excluding the very northernmost Lapland, the intermediate seasons would become longer, and the effective temperature sum would have a 1.5–fold increase (Ruosteenoja et al. 2014). According to one model, the conditions even in Lapland would eventually resemble current conditions in southern Finland (Ruosteenoja et al. 2014). Already, in the past 130 years' time, the phenology of ten bee species in North America has advanced by approximately ten days (Bartomeus et al. 2011), but on the other hand, at least generalist plant species seem to have advanced their phenologies accordingly. Significant changes in temperatures combined with an increased frequency of heat waves (Meehl & Tebaldi 2004) could dramatically affect the local Finnish bee fauna, causing changes in species distributions and most probably decrease their numbers dramatically, and a vast number of species would become endangered.

1.2. Bumblebee Biology

1.2.1 Natural Bumblebee Populations

Bees originated more than 70 million years ago around the Cretaceous era (Evans 1969). They have evolved together with the angiosperms to form the present fauna of approximately 25,000 described species in over 4,000 genera. Eusociality in bees has evolved at least eight times via different routes, and one of the five major social insect groups is the Bombinae, to which bumblebees belong. Bumblebees (Hymenoptera, Apoidea) have an almost as complex social organization as the well-known honeybee (Goulson 2010). Approximately 250 species of bumblebees, including the few inquiline cuckoo species (formerly categorized into their own genus *Psithyrus*), belong to one genus (*Bombus*) (Cameron et al. 2007). Northern species have an annual life cycle, which begins with one queen founding a colony of workers and then generating offspring (Sladen 1912, Alford 1978). Some species, such as *B. terrestris*, are highly flexible in their phenology. In areas of mild climate, they are able to maintain active colonies throughout the winter (Cumber 1949, Ferton 1901, Sladen 1912) and even achieve high foraging rates (Stelzer 2010). Bumblebees are haplodiploid, which means that the

females are diploid and are produced through meiosis, but drones hatch from unfertilized haploid eggs and can be produced also by workers later in the colony cycle. The queens and female workers are visibly identical, but the new queens weigh more because they have more fat in their abdomen. This extra fat is needed for winter diapause (Richards 1946, Cumber 1949). The bumblebee colony grows all summer until sexual offspring (new queens, i.e., gynes, and males, i.e., drones) have left the nest to find their mates. Workers, drones, and the foundress queen die eventually, and only new queens survive through the winter until next year when the colony cycle begins again.

Bumblebees have a unique thermoregulation system that allows them to generate metabolic heat and maintain their body temperatures above the ambient temperature, which again allows them to forage in cooler temperatures than, for example, honeybees (Heinrich 1996). With this endothermic ability, they are larger than average size when compared to most other insects, and with their insulating layer of hair, bumblebees are well-adapted for living in cool environments (Heinrich 1992). Female bumblebees are able to heat up their abdomen and use it to incubate the brood (Heinrich 1979) and the worker caste maintains the within nest temperatures by both warming and fanning to cool the nest (O'Donnell & Foster 2001).

Bumblebees are most abundant in the northern hemisphere (Goulson et al. 2008). Although there are some oligolectic bumblebee species that are entirely dependent on one species or genera of flowers (such as *B. consobrinus* that uses solely the rare monkhood [*Aconitum septentrionale*] in Finland), most bumblebees are rather generalists in their floral preferences (Goulson et al. 2008). The floral choice range of different bumblebee species depends partly on the length of their tongue. Nectar robbing species exist, which bite a hole into the flower to reach the nectar, but otherwise the short-tongued bumblebees are not able to use flowers with deep corollas. Flower preference varies between species, and some tend to visit sparsely distributed flowers more often than those with a dense distribution (Teräs 1985). Bumblebees have evolved to be very efficient in pollination by using a highly organized foraging system to gain floral rewards (for example, Wells & Wells 1986). In addition to being versatile pollinators with adequate worker numbers, bumblebees are not as aggressive as most other stinging bees and have become very popular in commercial use, especially for the pollination of greenhouse tomatoes.

1.2.2 Commercial Bumblebees

Commercial bumblebee rearing started in the 1980s in the Netherlands when the efficacy of bumblebees as pollinators of greenhouse tomatoes was discovered (Goulson 2010). Only three years after the commercial strains of the buff-tailed bumblebee *Bombus*

terrestris became available, 95% of the tomato growers in the country were using them instead of honeybees or hand pollination, and they very rapidly gained a standard status in pollinating greenhouse tomatoes in Europe (Goulson 2010). Presently, their use has spread all over the world, e.g., North Africa, Japan, New Zealand, Korea, and Russia. Few countries, including Norway, Canada, and the United States, banned the import of foreign bumblebees, and instead, either allowed the import of species naturally occurring in the country, or initiated the commercial production using their native bumblebee species. Commercially produced bumblebees are currently mainly used in greenhouses, but also to enhance the yields of several soft fruit crops in open fields and polytunnels (Velthuis & Van Doorn 2006).

Commercial strains of *B. terrestris* are used in Finland in greenhouses to pollinate mostly tomato and other vegetables and at orchards for apple and pear pollination. *Bombus terrestris* was identified for the first time in 1994 in Finland (Pekkarinen & Kaarnama 1994), but it remains yet unknown if the species has dispersed naturally or *via* the imported colonies. In 2009, the Finnish Forest Research Institute published a report about an experimental research using *B. terrestris* outdoor nests to pollinate bilberries (*Vaccinium myrtillus*). The yield in areas with enhanced bumblebee pollination was 12 times higher than the national mean in common open fields (METLA 2009). Over 200,000 imported bee and bumblebee individuals (bumblebee numbers are not separately reported by the Finnish Food Safety Authority, Evira) to Finland yearly shows a wide interest in using commercial pollinators, and their use will most probably increase in the future. Thus, the opportunities for non-native pathogens and other disturbances linked to commercial pollination are likely to become more common in Finland, which further increases the need for efficient monitoring and controlling systems.

1.2.3 Social Organization

Social insects, especially ants, weigh more in biomass and consume more energy than vertebrates in most terrestrial habitats (Wilson 1971). The degrees of social behavior in insects varies from subsocial colonies to highly organized societies (for one classification of social stages, see Michener 1969). Eusocial insects, such as termites, ants, and some of the bees and wasps, belong to the highest category of sociality. In this category, they have separate reproductive castes and a partially or wholly sterile worker cast. The non-reproductive or less reproductive worker individuals cooperate in taking care for the offspring, and they have overlapping adult generations within the colony. Cooperation, and the reciprocal communication it involves, can be viewed as the essential criterion of sociality (Wilson 1971). Eusocial insect colonies are often referred to as superorganisms, because the result of their cooperative behavior can be seen as

analogous to the physiological functions of different organs or tissues (Wheeler 1928, Wilson 1971, Hölldobler & Wilson 2009).

The study of social insects and their behavior started growing in the late nineteenth century when evolutionary philosophy arose and the levels and orders of the world were questioned (Hölldobler and Wilson 2009). Myrmecology, the study of ants, has been the basis for all social insect studies. Between the 1950s and 1970s, research concentrated on topics such as chemical communication, caste system evolution, and the physiological factors of caste determination. Sociobiology, having social insects as its centrum, was established during the 1970s and 1980s. More recently, new areas such as sociogenetics and sociogenomics have appeared, but still systematics and the scientific study of the natural history of social insects persist firmly in the background.

Studies conducted during the past 20 years have revealed that unlike previously thought (e.g., Dornhaus & Chittka 1999), bumblebees have a very complex social system. In most bumblebee species, the size distribution of females is bimodal and there is hardly any overlap between queen and worker castes, but otherwise they are structurally identical in their external morphology (Goulson 2010). Queens, however, have significantly more fat in their abdomen, in contrast to the workers, whose abdomen is capable of storing more honey. There are several hypotheses of how queen caste of bumblebees is determined, but at least in *B. terrestris*, this happens early during the larval stage through pheromonal cues secreted by the queen, to which larvae are sensitive, and which control their juvenile hormone production (Röseler 1970, 1991, Cnaani et al. 1997, 2000).

Size variation is substantial in bumblebee workers indicating an adaptive function of having a large size range within a nest (Goulson 2010). Behavioral specialization, i.e., polyethism (functional specialization), is not as clear as in honeybees, because unlike honeybee workers, bumblebee workers often switch between foraging and nest maintenance tasks (Free 1955, Van Doorn & Heringa 1986). Young workers always start by maintaining the nest, but some switch to foraging when they get older (Povreau 1989, O'Donnell et al. 2000, Silva-Matos & Garofalo 2000). Larger workers tend to switch to foraging earlier than smaller workers, but the smallest workers usually stay in the nest all their lives (Povreau 1989). In large nests, there are also guard bees that inspect foragers at the nest entrance (Free 1958), and this task is often carried out for several days by the same individual (Goulson 2010).

1.2.4 Queen-Worker Conflict

Due to the haplodiploid nature of bumblebees, the relatedness of workers in the nest is greater than that of a worker and its progeny in species where female mates with one

male. The relatedness of full sisters is 0.75, whereas the number is only 0.5 with their own (male) offspring. This phenomenon has often been viewed as a basis in the evolution of sociality in Hymenopterans, because it gives a plausible explanation to their social behavior. The theoretical background for this idea is presented by Hamilton (1964). According to Hamilton's rule (r B > C), helping others will sustain in a group when an individual's profit from helping its relatives (B) multiplied by the degree of relatedness (r) is greater than an individual's disadvantage (C). Thus, if related individuals share the benefits, co-operation can be stable, because relatives are more likely to carry the same set of genes than any random individual in the same population. The higher the coefficient of relatedness (r) is, the higher the probability of shared genes among a population. Kin selection (Hamilton 1964, Smith 1964, Price 1970) as an evolutionary strategy favors the success of relatives in a group and assumes that individuals benefit from helping one another even at the cost of their own reproduction or survival.

As long as the colony is growing, it is more profitable for bumblebee workers to help in rearing their siblings than it is to reproduce own offspring. However, towards the end of colony maturation when the reproductive progeny is produced, some workers become increasingly aggressive and their ovaries start to develop (Free 1958, Foster et al. 2004). These workers may produce unfertilized (male) eggs, which are often eaten by the queen (Free et al. 1969). Workers have also been observed eating the eggs laid by the queen (Van Honk et al. 1981, Duchateau & Velthuis 1989, Bloch & Hefez 1999). This conflict between the queen and workers has been suggested to result either when the queen loses her dominance in the nest due to oppression by the workers (Duchateau & Velthuis 1988, Röseler 1991, Ansalem & Hefetz 2011) or because of the queens own volition (Bourke & Ratnieks 2001).

The timing of the reproductive phase of bumblebees has been studied mostly in *B. terrestris*, which belongs to the same group as the native study species in this thesis, *B. lucorum*. The time at which the colonies switch to rearing reproductive offspring varies among colonies: 50 percent of colonies have their switching point approximately ten days after the hatching of first workers, the other half switch about 24 days after first workers emerge (Duchateau & Velthuis 1988, Duchateau et al. 2004). Early switching colonies tend to produce mostly males in *B. terrestris*, whereas late switching colonies produce mainly new queens (Duchateau & Velthuis 1988). In both colony types, the competition point where workers begin reproducing starts 31 days after the first worker progeny has emerged (Duchateau & Velthuis 1988).

According to the kin selection theory, monogamous queen colonies should produce females to males in 3:1 ratio (Hamilton 1964, Smith 1964, Price 1970). However, most

bumblebee populations have a heavily male-biased sex ratio (reviewed in Bourke 1997). There are several hypotheses why bumblebees tend to produce more males than females. First, males are cheaper to produce because they are markedly smaller than queens. Second, workers reproducing males could potentially lead to male-bias. Third, if males emerge before the new queens (protandry) and especially when the male production is controlled by the queen (Bulmer 1983), the resulting sex ratio is often male-biased. Another possibility creating male-bias could be that after a certain time point, the queen loses the benefit of laying eggs that develop into new queens, because they take longer to develop and need to be out from the nest early before the end of the season. This is because new queens need time for mating and digging their hibernating chambers. Thus, later in colony development, when the optimal number of gynes have been reared, both workers and the queen should invest in their own male progeny and the queen should use all of its remaining resources into male production. Additionally, because the survival of new queens through winter hibernation is linked to their size (Beekman & Van Stratum 1998, Vesterlund et al. 2014), male dominance in sex ratio could be the optimal strategy during low resource availability (Beekman & Van Stratum 1998). However, none of the explanations has yet fully explained this phenomenon.

1.3. Bumblebee Conservation

1.3.1 Strategies and Monitoring

A vast number of native flowers and important crop species are dependent on bumblebee pollination, and decline in their numbers is bound to have negative effects on the abundance of these species. One implication might be, for example, reductions in seed set, which could lead to inbreeding through reduced outcrossing. Reductions in the species richness and abundance of bumblebees may cause plant communities in large areas to change (Corbet et al. 1991). The yields of many commercially important crops are increased using bumblebee pollination services (Free 1993, Osborne & Williams 1996, Carreck & Williams 2002). Bumblebees are essential contributors to our natural capital by providing food security and the maintenance of wider plant biodiversity. Therefore, their conservation should be high on the list of priorities in every country, especially if its economy is based on agriculture.

Hedgerows, uncropped field margins, tussock strips across fields, and flower rich grasslands nearby (Kaule & Krebs 1989, Firbank et al. 1993, Marshall et al. 1994) encourage bumblebee diversity. These environments increase the diversity of flowering plants and provide nest sites for bumblebees and many other pollinating insects. In a typical Finnish farmland, the species richness of bumblebees is highly correlated with

the abundance of zigzag clover, *Trifolium medium* (Bäckman & Tiainen 2002). Assigning a conservation status for endangered cryptic species may be a huge challenge and many species certainly go unnoticed and disappear without ever being acknowledged. Monitoring bumblebees is essential in gathering all needed information for their protection. Conservation of targeted species is only possible with adequate information about species abundance and distribution. The need for conservation cannot be noticed without knowledge of the possibly endangered species.

A monitoring system of Finland began in 1997 to follow long-term changes in the pollinator communities of grasslands and forests of Northern Europe (Söderman 1999). A pilot monitoring was performed using yellow colored funnel traps that were installed in clusters of three and hung one to two meters above ground and three to five meters apart, along a forest margin. Yellow traps showed promise for pollinator monitoring, but Söderman (1999) concluded that the method worked better for the estimation of bumblebee queens than of workers or drones. It is important to continue the development and testing of alternative monitoring methods, because conservation strategies based on actual distributions and abundances are more efficient than untargeted general conservation methods.

In the United Kingdom, several bumblebee species are already extinct or highly endangered mainly because of habitat loss and population fragmentation. After not being found for 14 years, *B. subterraneus* was declared extinct in 2002, and, for example, *B. distinguendus* and *B. sylvarum* are rapidly declining (Goulson 2008). To enable the recovery of the surviving small and isolated populations, targeted habitat management must be promoted. This includes habitat restoration and favorable management *via* agri-environment schemes within a 10 km radius of the endangered species populations (Goulson et al. 2011). For the extinct species, there are few possibilities. Occasionally species that are only locally extinct may be reintroduced and, for example, the formally extinct *B. subterraneus* was reintroduced to the UK from Swedish source populations (The short-haired bumblebee project 2013).

1.3.2 Species identification

Inaccurate or false identification can be a serious problem, especially if rare species remain unnoticed among widely distributed common species. It is not always very easy to draw the line between different taxa, and researchers have long debated over what defines an actual species. There are several species concepts that differ from one another in their perspective. One of the most widely used is the biological species concept (first formed by Ernst Mayr in 1942), which states species being “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from

other such groups." Mayr later (in: Mayr & Provine 1980) refined this into the idea that species are reproductive communities of populations that are reproductively isolated from others and occupy a specific niche in nature. The mechanisms of reproductive isolation, however, differ among taxa, and the biological species concept offers no universal means to delimit species. Detailed methods within taxa are therefore always needed to discriminate species.

Ridley (1993) challenged the biological species concept by introducing a cladistic one, where species are considered as an evolutionary lineage of organisms: "between two branch points or between one branch point and an extinction event or a modern population." He also suggested that the concept ecological species (term first defined by Van Valen in 1976) is a set of organisms exploiting or adapted to a single niche, and that the ideal definition of a species would be a combination of biological, recognition, ecological, and cladistics concepts (Ridley 1993). The same year (1993), King concludes biological species concept to be the best choice out of eight different possibilities. However, as Mallet (1995) explained creating his Modern Synthesis for species definition, within-species polymorphism and racial variation between geographical areas challenge the biological concept derived originally from the work of Darwin (1859). Mallet (1995) speaks for the use of genotypic clusters, a definition used by many systematics: species, or taxonomic units, can be separated from one another when there are few or no intermediate forms (at single and multiple loci) when they are in contact, and the individuals of groups can be divided into genetically identifiable clusters using patterns of difference as cues to detect them.

In this thesis, I use a combination of the biological species concept and genotypic cluster definition, bearing in mind that the line between species can be vague, and evolution is constantly shaping the boundaries and often resulting in a gradient of species very closely related to one another.

1.3.3 Cryptic Species

When two or more species are biologically distinct, but morphologically indistinguishable, and are often viewed as a single species, they are defined as being cryptic. In cryptic species, the morphological changes are often so subtle that they remain undetected by traditional taxonomy and usually such species can be separated from one another only by molecular methods such as DNA sequencing or polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) (Bickford et al. 2007). If two species in a cryptic species complex have only recently, during the historical timeframe of biology as a science, been separated from each other, and can be determined as closest existing relatives, they are called sibling or sister species (Knowlton 1986).

Cryptic species usually utilize nonvisual mating signals, which promotes morphological statis, i.e., stabilizing selection that reduces morphological changes usually associated with speciation (Bickford et al. 2007). Bumblebee males and queens use species-specific sex pheromones secreted from their labial glands to attract and find mates (Kullenberg et al. 1973, Bergström et al. 1981, Krieger et al. 2006). The distribution and classification of bumblebees is complex. The subgenus *Bombus* (*s. str.*) is widely distributed in the holarctic area, but the actual number of species is not resolved because of existing, subtle, intraspecific variation. One of the most common species in the subgenus, the white-tailed bumblebee (*B. lucorum*), has 186 synonyms thus far (Williams 1998). Five species belonging to *Bombus* (*s. str.*) subgenus are present in Finland: *B. cryptarum*, *B. lucorum*, *B. magnus*, *B. terrestris*, and *B. sporadicus*. The first three species are often called the *B. lucorum* group or complex because they are not easily distinguishable from one another in field surveys and the observations of these species are often presented as one group. In Finland, *B. terrestris* is often excluded from these classifications because of its undermined existence there and difficulties in distinguishing its workers morphologically from the *B. lucorum* group. Therefore, previous field surveys have likely overlooked the species despite the fact that it has been used commercially in Finland for over 25 years (information given by commercial bumblebee retailers). During a pilot monitoring of pollinators (years 1997-1998), no *B. terrestris* were captured in Finland, but 320 individuals were caught from several locations in other countries in Eastern Fennoscandia and the Eastern Baltics (Söderman 1999). The last species of the subgenus, *B. sporadicus*, is easily distinguished by its morphology.

1.4. Aims of the Thesis

I. My first aim was to study the cryptic *Bombus s. str.* species complex by identifying all specimens of the trapping samples gathered from the transect lines in south-western Finland and to modify existing molecular methods to be able to use short fragments from degraded DNA for bumblebee identification to facilitate the risk assessment of bumblebee species in this group.

II My second aim was to enhance bumblebee monitoring methods and to determine the abundance of *B. terrestris* in two locations in Kemiönsaari, Finland. My sub-aim was to study if the commercial use of *B. terrestris* increases the possibility of finding this species in nature near tomato greenhouses and apple orchards.

III My third aim was to reveal how native *B. lucorum* reacts to different winter temperatures, especially high temperatures predicted by several climate change scenarios. The threshold weight for survival, phenoloxidase activity after diapause,

and fat expenditure during four months of overwintering was compared for two temperature groups (9°C and 1.8°C). Most of the previous work on the subject has been done using commercially reared *B. terrestris* and one side-aim was to determine if *B. lucorum* reacts differently or similarly to *B. terrestris*.

IV My fourth aim was to study the effect of queen size and worker population on the production of reproductives and colony characteristics of the native white-tailed bumblebee *B. lucorum* using laboratory reared colonies.

V My fifth aim was to assess how high spring temperatures affect the survival of bumblebee queens during food scarcity compared to the normal mean temperature in Finland. Changes triggered by climate change, such as phenological asynchrony between flowers and insects or unusual frost damage of spring flowers may create a situation where queens may not be able to find food when they emerge from diapause in the spring. Newly emerged *B. lucorum* queens were experimentally starved at two temperatures (15°C and 24°C) and the number of phoretic mites on them was hypothesized to signal their overall condition.

There are three bumblebee species within the five most important pollinators of European crops (The IUCN Red List of Threatened Species 2014). Because of their economic value in food production and their importance for natural flower pollination, the experimental research to understand how bumblebees react to high temperatures, and what their physiological limits are, is vital. The results of this thesis will shed new light on how bumblebees are affected by climate change-related temperature changes as well as present information and methods essential in the conservation of native bumblebee populations in Finland.

2. MATERIAL AND METHODS

2.1. Study Area (I, II, III)

I conducted this study in Kemiö, the largest marine island in Finland excluding the main island of Åland. Kemiö is situated in the latitude corresponding with the Swedish distribution of *B. terrestris* and this species is used both in greenhouses and in apple orchards for pollination in different locations of the island. I trapped bumblebees for two consecutive summers, 2008 and 2009. In July 2008, eight yellow window traps were based on the ground (ground trap) and eight in the trees (tree trap; Fig 1b) in two modified transect lines in Kemiönsaari, making a total of 32 traps (Fig. 2). The first 16

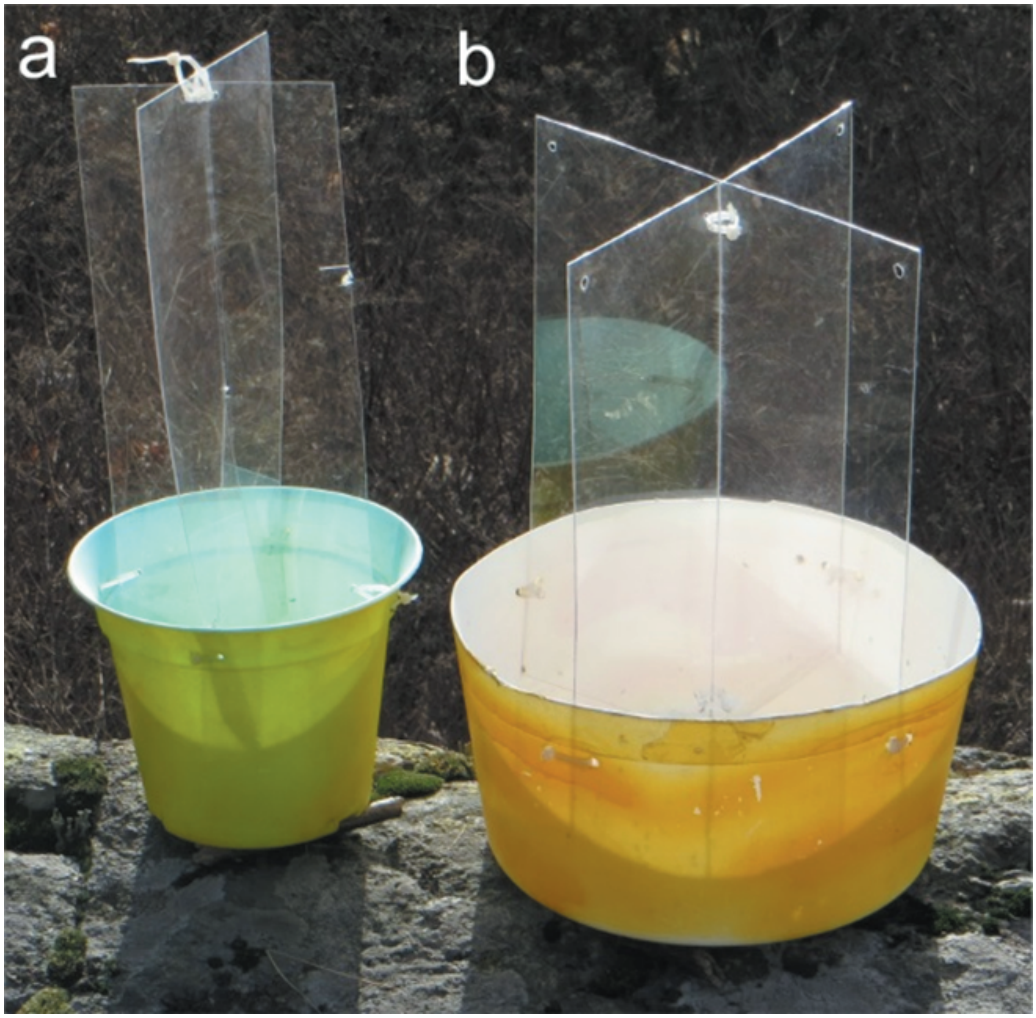

Figure 1. Luring yellow traps. - a. Crown trap. - b. Tree trap.

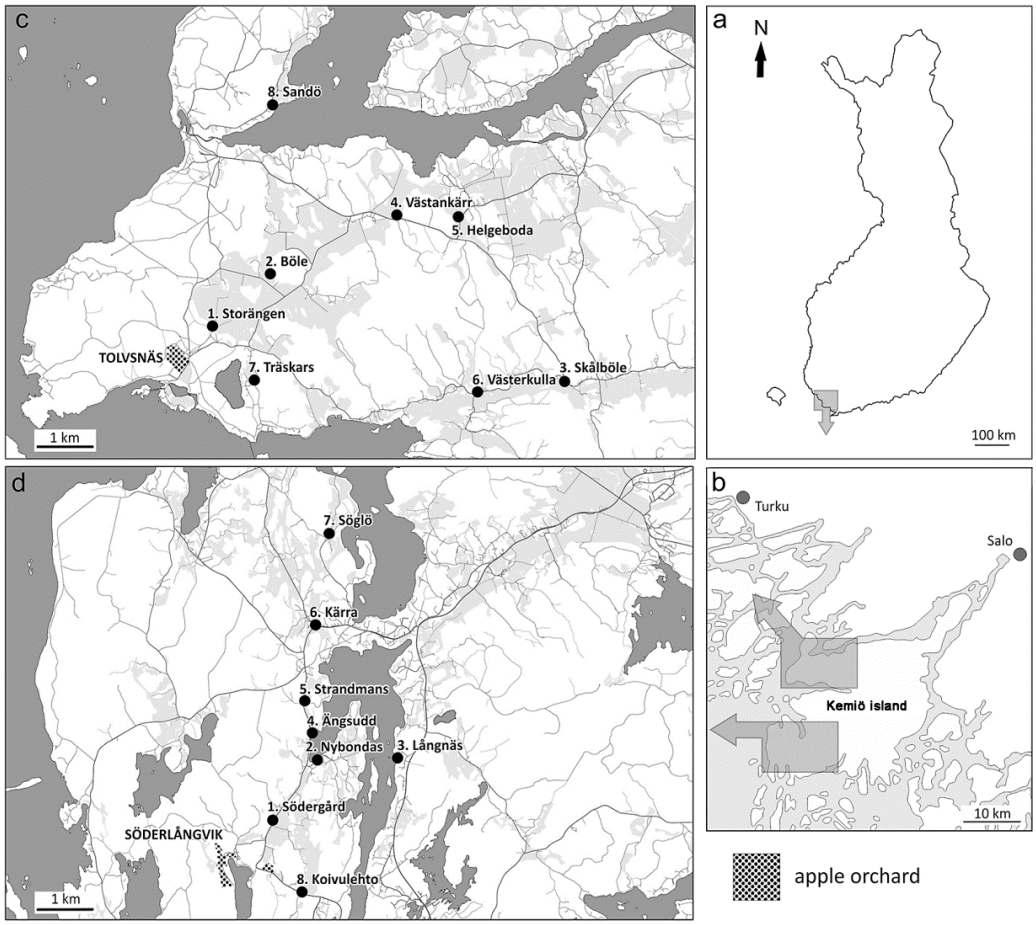

Figure 2. The modified trapping places of the transect lines of Tolvsnäs and Söderlångvik in Kemiönsaari, SW Finland. The trapping areas were situated to follow the expected dispersal routes at 1–6 km distance from greenhouses and/or orchards.

traps were located around a 12 hectare apple orchard with approximately 7,700 trees in Tolvsnäs situated in a one to six kilometers distance from the center of the orchard, where the imported bumblebee nests are held. The second trapping area was approximately 15 kilometers south from the first one, around an 11 hectare apple orchard and a large tomato greenhouse of Söderlångvik manor. The latter traps were situated in a one to six kilometers distance from the greenhouse. Both sets of traps followed modified transect lines, which were drawn on the map in the most probable dispersion directions according to the landscape patterns of the area.

I expected the main dispersion route to be to the North or North–East in both places due to the presence of the sea in the East and South of these areas. I sampled the traps at seven to 14 day intervals between July and August, 2008. During the next spring, I added a third trap (crown trap; Fig 1a) approximately five meters high to accompany

the other two traps. I collected bumblebees from all traps between June and August, in 2009. I initiated trapping in all 16 sites earlier than the previous year, before the hibernated queens started to fly and seek nest sites, to measure the possibility of successful *B. terrestris* hibernation in nature. In addition to the trapping samples, a total of 23 *B. terrestris* specimens (10 workers, two drones, and 11 queens) were collected by hand or as by-catches during insect trapping from various locations in South-western Finland by a local bumblebee rearer, Erkki Kaarnama (Forestum Oy), and senior museum technician Anssi Teräs (Åbo Akademi University and University of Turku) during 2009 to 2011. These specimens I used in the development of a new method but also as observational data to show, that *B. terrestris* individuals can quite easily be found from nature in Finland, and trapping can only give a guideline of the true density relative to other similar bumblebees.

2.2. Identification of Bumblebee Species (I, II)

Bumblebees gained from the traps were first identified to species level using morphological characters. We used the keys of Benton (2006) and Söderman and Leinonen (2003), as well as the Swedish field identification tool of Björn Cederberg (ArtDatabanken 2009) for the identification of all samples. For the cryptic *B. lucorum* group and *B. terrestris*, I used also the genital characteristics of queens described by Pekkarinen and Kaarnama (1994). Many specimens were damaged during trapping and collecting, and were often impossible to identify on the species level. The DNA was also deteriorated in many specimens, which meant that earlier methods using long (more than a thousand base pairs) DNA-fragments did not amplify well. Therefore, we developed a new method that uses short (fewer than 500 base pairs) DNA-fragments and is able to separate all species, excluding one Irish haplotype of *B. cryptarum* that has to be verified by sequencing to separate it from *B. magnus* (I). During the development process, I discovered nuclear mitochondrial DNA (NUMT) in bumblebees, which is very important for the accuracy of molecular methods, because the co-amplification of NUMTs may cause incorrect species identification (Moulton et al. 2010). This knowledge is essential to avoid errors in future use of rapid molecular methods such as PCR-RFLP or barcoding.

Individuals belonging to the subgenus *Bombus s.str.* (*B. terrestris*, *B. lucorum*, *B. cryptarum*, and *B. magnus*) were identified using a modified PCR-RFLP-method for degraded DNA. New primers, forward 5'-GAAACCTTTGGAAATTTAAGA-3' and reverse 5'AATTGAATTTTTAATCATTTTTGA-3' were designed for amplification. I used a MJ Research PTC-100 Peltier thermal cycler for amplification with a protocol commonly used with Qiagen multiplex PCR kit (see article I). This approach uses a short (446 bp) partial mitochondrial COI fragment and three diagnostic restriction enzymes

(Hinf I, Hinc II, and Hae III) separately, in a stepwise manner. The restriction products of each enzyme were separated on a 1.5% agarose gel. To validate the method, a subset of samples (N=96) were sequenced using Sanger sequencing (Macrogen Inc., Republic of Korea) and compared with PCR-RFLP results.

2.3. Bumblebee Rearing (III, IV)

I captured overwintered queens of *B. lucorum* (N=140) from four locations in South-western Finland (three in Turku, one in Kemiö) during late April to early May 2011. I trapped the queens soon after their emergence when the daily temperatures allowed for flight. I used glass jars and plastic 50 ml tubes to trap the queen bumblebees by hand. I observed the queens when they searched for food and caught them straight from flowers such as *Crocus* sp. when they were feeding. Only queens not carrying pollen were used to avoid capturing individuals, which may have already initiated egg-laying. Capturing was also timed very close to the emergence for the same reason.

I reared the queens in a laboratory under a red light (non-visible to bumblebees) at 24-25°C temperature and 50-60% relative humidity. Bee-collected, fresh frozen pollen and a BioGluc-sugar solution (Biobest, N. V., Belgium) were provided *ad libitum*. Each queen was placed into a box made out of a round 0.25 liter clear plastic container (diameter: 10 cm, height: 12 cm) held upside down with its lid as the bottom. Holes were drilled into the lid following the edge line to help keep the box clean from faeces. A hole (5 x 5 cm) was cut in the bottom of the container (the top of the queen box) and a piece of 2 millimeter stainless steel wire mesh was glued on with warm glue to allow feeding and ventilation. For additional ventilation, holes (2 mm in diameter) were drilled around the container walls. A piece of cardboard was taped in the bottom for keeping the container clean and dry. The rearing was initiated by placing a pea-sized pollen ball (moisturized and formed using nectar) into the initial rearing box. The pollen ball was changed into a new one if the queen broke it or it dried out.

Most queens (N=131) started laying eggs. After or right before the first workers hatched, the developing colony was moved into a larger nest-box. The box (30 x 40 x 40 cm) was made out of veneer plywood and 2 mm stainless steel wire mesh with a removable clear plastic lid on the top. Nectar was provided with a commercially sold feeder for bumblebees (BioGluc by Biobest N. V., Belgium) and fresh pollen was also given continuously. The nests were monitored until they started producing sexuals (new queens and drones) or until the workers started to die, and all cocoons had hatched. Of these 131 nests, 34 nests produced queens (1-6 queens, each) with a total number of new queens of 92. The new queens were put in separate boxes and their nest of origin was recorded. A week

after hatching, new queens were placed, one at a time, in 60 x 60 x 60 cm mating cages made out of 2 mm stainless steel wire mesh and wooden frames. A variable numbers of drones from one nest was used at a time and never from the same nest as the queen. The cages were continuously observed and if the mating had not started within 30 minutes, the queen was put back into its box. Each queen was tried with different drones, up to 10 times, before marking it unfertile / non-compatible. 63 out of 92 queens (68%) mated with drones from other reared nests and were used in the overwintering experiment.

2.4. Diapause Experiment (III)

I divided the queens into two treatment groups (A and B) in the order of their mating, such that there was an equal number of sister queens in both treatment groups, except when their numbers where not equal. Both groups were acclimatized without feeding at constant darkness at 15°C for two weeks before the start of the four months diapause experiment. I weighed the queens before starting the diapause treatment and placed each queen in a lightly closed 50 ml plastic tube. In the tube, there was a cotton ball moistened with 2 ml of deionised water on the bottom and above that a 7 cm layer of vermiculite-perlite-mix, and finally, the queen on the top. Group A was put to diapause at cold (0 to 3°C) and group B at warm (7 to 10°C) conditions. I checked the survival and weighed the queens regularly during the four month diapause.

2.4.1 Physiological Measurements

After four months of hibernation, the queens were awakened in 20°C and then chilled on ice before taking the hemolymph samples. Hemolymph was needed for phenoloxidase (PO) activity measurements. The baseline levels of the PO activity were used to measure the differences in *B. lucorum* queen responses after overwintering. The total protein concentration in the hemolymph was quantified using the Bio-Rad (Bio-Rad Laboratories, Helsinki) protein assay kit based on the Bradford method (Bradford 1976).

Body fat content was used to quantify the nutritional status of queens (e.g., Sundström 1995, Hahn 2006, Sorvari & Hakkarainen 2009). I removed the honey-filled stomach by carefully cutting off the thorax. I then inserted the abdomens into individual test tubes and put them into -21°C until they were later freeze-dried for 48 hours. The abdomens were weighed on a precision scale after which 8 ml of petroleum ether was added to the tubes and they were placed in an ultrasonic bath for 4 hours. Petroleum ether was used instead of chloroform because bumblebees are extremely hairy, and chloroform tends to melt the hair. Petroleum proved better with this species when we compared both substances prior to using our own specimens. After the ultrasonic bath, the samples were rinsed twice for 10 minutes with 8 ml petroleum ether. The abdomens were then placed

in a drying oven at 45°C for 48 hours and weighed. The difference in dry weight before and after petroleum ether treatment is interpreted as the amount of fat dissolved from the individual during the process (Sundström 1995, Sorvari & Hakkarainen 2009). The fat-free dry weight of the abdomen is used as a measure of the size of queens in the analyses of body fat and immune parameters.

2.5. Sex Allocation (IV)

The colony development of all nests reared in order to produce new queens (for study III) was recorded during the rearing period to measure variables which may influence the sex allocation of produced colonies, such as: maturation time, egg-laying delay, worker population size at different time points, number of gynes and their weight, number of drones, and foundress queen weight. The nests were checked a minimum of three times a week from when the queens were placed into initial rearing boxes until the sexual progeny was produced and used for mating. The final check was done after the colony had seized reproduction and was terminated.

2.6. Spring Starvation Experiment (V)

I collected *B. lucorum* queens (N=80) from various locations in Southwestern Finland during the 22nd April to 1st May, 2012. Of these, I used 77 queens in the analyses and excluded three due to their drowning in the water dispenser (model commonly used in bird cages with a 100 ml plastic water dispenser outside and a 1 cm wide and 2 cm long drinking area inside the cage). The queen box was the same model that was used in bumblebee rearing. Usually the presence of phoretic mites on the body of bumblebees is thought to be harmless, but it may become harmful when the number of mites increases to hundreds or even thousands per individual bee (Chmielewski & Baker 2008), because movement and flying becomes extremely hard. In addition, a high number of mites may indicate a poor body condition of the queen. I counted the number of *Parasitellus fucorum* mites from each queen using three classes of estimated parasite numbers: 0 = no visible mites, 1 = 1 to 70 mites, and 2 = greater than 70 mites. I counted the mites in the field immediately after capturing the queen. The escape of mites was controlled with insect control greenhouse fly traps (Silvalure, Silvandersson Sweden AB), which I placed under each individual queen's box.

I divided the queens into four groups taking into account their initial load of *P. fucorum* mites: (A) 15°C and provided with only water, (B) 24°C and provided with only water, (C) 15°C and provided with pollen and nectar, (D) 24°C and provided with pollen and nectar. The survival of queens was checked once a day until all the queens in groups

A and B were dead. The queens provided with food (group C and D) were held in the experimental setup for 25 days before the removal into rearing facilities, where they were reared until they produced eggs. After or near the hatching of their first workers, I released the queens back to nature in wooden nest boxes. Small specimens are likely to have less fat in their abdomen. Because of the potential differences in the responses of queens with different weights, I measured the fresh weight of queens and used it as a covariate in the data analysis.

3. RESULTS AND DISCUSSION

3.1. Identification of Cryptic Species (I, II)

The first aim of my thesis (I) was to study the cryptic *Bombus s. str.* species complex, which includes an interesting introduced species to Finland, the buff-tailed bumblebee *B. terrestris,* which is used commercially to pollinate various fruits and crop species. This species is generally of more southern origin than the native bumblebees of the same subfamily, and has proven to be more flexible in its requirements and a very strong competitor (for example, Ings et al. 2005, Stelzer 2010). *Bombus terrestris* is often confused with the local white-tailed bumblebee, *B. lucorum*, and its sister species, *B. magnus,* and *B. cryptarum*. These cryptic species are extremely hard to differentiate unless they are identified by a specialist in bumblebee identification, and these experts are few and not always available. The different trapping types and variable weather conditions, during monitoring, can also damage the samples, making some identification methods impossible.

For being able to assess this species group, we developed a new PCR–RFLP approach by modifying earlier methods (Murray et al. 2008). The new modified method (Chapter I) is most useful in distinguishing *B. lucorum* and *B. terrestris*, but it also enables separation of most *B. magnus* and *B. cryptarum* specimens. From 863 trapping samples (I), total DNA was extracted from 810, of which, 667 (82.3%) were successfully amplified with our method. Of these, four samples were identified as *B. terrestris*, nine as *B. cryptarum*, none as *B. magnus*, and the majority (654 samples) as *B. lucorum*. Two *B. terrestris* samples, later verified by sequencing, were initially correctly identified using morphological characters. The other two were first misidentified as members of the *B. lucorum* complex. There were 646 samples belonging to the *B. lucorum* complex, identified based on the PCR-RFLP-approach, while only 586 were correctly identified (to the complex level) using morphological characters (Söderman & Leinonen 2003, Benton 2006, ArtDatabanken 2009). This means that as many as 60 individuals (9.2%) were misidentified prior to using genetic methods. The new PCR-RFLP protocol we developed (I) is especially useful for identification within the problematic *Bombus s. str.* species complex when sample DNA is degraded, with old museum specimens or when specimens are damaged by various liquids used in traps, and only short fragments can be amplified. With this method, we were able to identify individuals in the trapping data as well as verify the species of all reared queens for later studies.

3.2. *Bombus terrestris* in Finland (II)

My second aim was to enhance bumblebee monitoring methods and to determine the abundance of *B. terrestris* in Southern Finland. Bumblebee pollination is highly valuable and monitoring their numbers is needed to protect this indispensable component of biodiversity. Contrary to my expectations based on literature and observational data from Southern Finland (23 *B. terrestris* specimens: 10 workers, two drones, and 11 queens), only four *B. terrestris* individuals were trapped during two field seasons. However, the traps worked well in capturing bumblebees in general (II, see also Kakko 2013), and this method can be recommended and further used in bumblebee monitoring. From the three, simple yellow trap types were used, varying in their size and placement, the most efficient trap for bumblebees was the 2 liter tree trap (trapping surface area was 0.26 m^2) placed at approximately three meters high (Fig. 1b). Because of the low number of trapped individuals of *B. terrestris* during two years' time it seems that commercial use has not markedly increased the occurrence of this species in Kemiönsaari. However, the observational data indicates that *B. terrestris* may have become more common in Southern Finland and the situation should be investigated further.

According to the Status and Trends of European Pollinators project (STEP 2014) and the European Red List of Pollinators (2014), bumblebee populations are dramatically declining. Pollination is an essential ecosystem service, which maintains wild plant communities and agricultural productivity. Natural pollination services depend on both domesticated and wild pollinator populations, which may be affected by a range of recent and projected environmental changes. Monitoring the Finnish bumblebee populations efficiently provides a template to guide future global warming scenarios and the problems caused by commercial bumblebee importation, such as: pollination disturbance of native plants (Kenta et al.2007), competition with native bumblebees and also other pollinators (Hingston & McQuillan 1998, Inoue & Yokoyama 2006), the outcompeting of native conspesifics (Ings et al. 2005), and the dispersal of novel parasites (Goka et al. 2001, Thorp 2005, Cameron et al. 2011, Goulson et al. 2008, Murray et al. 2013).

Without knowing the current situation, it is impossible to detect if some species are in danger of becoming extinct or endangered. The status of the imported bumblebee in Finland should be investigated more thoroughly by widespread sampling, because it is now shown that the numbers of accounted *B. terrestris* in general have increased since its introduction. Although no connection was found between the distribution of *B. terrestris* and the proximity of the greenhouses or orchards, these could have served as the initial dispersal source. Ten years after the beginning of commercial imports in

Japan, the number of *B. terrestris* colonies used in greenhouses is no longer a limiting factor for the distribution of the species, implying that it had already been naturalized and that greenhouses were no longer main source populations (Kadoya et al. 2009). *Bombus terrestris* specimens were not found in 1997 in Finland during a two-year pilot monitoring of pollinators throughout Eastern Fennoscandia and the Eastern Baltics (Söderman 1999). A similar survey would most probably give different results with more *B. terrestris* observations if conducted now, after 25 years of commercial pollination and 19 years since the first record of the species in Finland.

The type of yellow tree traps used in my study would be one efficient and cost-effective way of monitoring local populations. A yellow colour is especially luring in spring when there are few flowers in sight, which is exactly why the use of yellow traps should be restricted to summer months, unless the purpose is specifically to catch queens. Trapping queens should be avoided in standard monitoring, because according to our trapping data, the yellow traps can catch hundreds of queens within a couple of weeks in spring. This could possibly negatively affect the local population by decreasing the number of queens able to start a new colony, and this way may skew the results many years onward.

3.3. Climate Change and Bumblebees (III, IV, V)

3.3.1 Diapause Temperature

My third aim (Chapter III) addressed the possible consequences of climate change related temperature changes, and how the native white-tailed bumblebee would react if subjected to higher than usual mean temperatures (Table 1). During the four-month experimental diapause, every queen under the pre-diapause weight of 0.4 grams died. This result was consistent with an earlier study by Beekman et al. (1998), where a similar threshold of survival was found for *B. terrestris*. The threshold weight of *B. lucorum* was 0.2 g lower than previously shown in this southern sister species (Beekman et al. 1998). The survival of *B. terrestris* queens has been shown to decrease with increasing duration of diapause even when durations less than four months were used (Gosterit & Gurel 2009). The survival of queens in this study was not otherwise analyzed because the experiment lasted only four months, which is significantly shorter than the normal winter diapause duration in nature in Finland (~7-8 months depending on the species), and further, because the experiment was not designed for measuring overwintering survival. Instead, I wanted to have as many queens alive after the diapause as possible for PO activity and fat analyses. The lower survival weight of *B. lucorum* was considered as a probable consequence of the overall size

difference between the two species, *B. terrestris* being on average the larger one. As all *B. lucorum* queens under the threshold weight died early on, it may be that they had already depleted some important part of their energy reserves, such as glycogen or the unsaturated fatty acids (Hahn & Denlinger 2011). Large queens survived better compared to smaller queens, but diapause temperature had no effect on survival during this relatively short-term experimental diapause.

The body-size corrected amount of body fat was generally lower in queens that overwintered in warmer temperature. Larger queens that overwintered in the warm treatment had proportionally more fat in their fat body after the diapause compared to smaller queens, undergoing the same treatment. The fat consumption difference between queens of variable sizes disappeared when the diapause temperature stayed lower/close to the normal mean. Metabolic rate correlates with insect body size but there is extensive variation in the metabolic elevation of these allometric relationships (Waters & Harrison 2012). The difference I observed seems to relate to a normal size-dependent metabolism rather than adaptive traits, although evolutionary differences in physiology and life history are likely behind some of the correlation patterns. On average, the queens used more fat in the warm compared to cold temperature, but since larger queens possess lower mass-specific metabolic rates, they had proportionally more fat left after the four month diapause than the smaller queens. Bumblebees are considered as having endothermic capacities for the regulation of their body temperatures using heat generated by their flight muscles (Heinrich 1992). Thus, it is likely that mild and long winters, and also prolonged growing seasons with warm autumns, would lower queen survival and indicate high energy expenditure because the queens are not able to reach full inactive diapause state until the temperature becomes cold enough. New queens also excavate in the late summer before the temperature decreases and their survival might be linked to the duration of cold versus warm periods during the whole diapause length.

Table 1 Positive, negative, and insignificant effects (marked with +, -, and 0, respectively) of warm temperature on queens in different size groups. A large size of queens had a positive impact on the amount of fat left after diapause, and there was no apparent negative effects of warm temperature on the immune parameters of large queens. Otherwise, an increase in temperature had a negative effect on the queens in both size groups.

	Queen size	
	Large	**Small**
Residual fat	+	–
Hemolymph proteins	–	–
Immune defence	0	–

Larger queens had a higher PO activity compared to smaller queens, but only in the warm diapause treatment. In the cold (mean: 1.8°C), which is considered as the normal overwintering temperature, there was no effect of size on the remaining fat in the fat body. However, queens had a higher hemolymph protein concentration, and this increase in concentration correlated positively with PO activity. PO-activity levels can be used as an indicator of the strength of the immune system, because of their vital role in maintaining immune functions (Dunphy 1991, Gillespie et al. 1997, Reeson et al. 1998). Castella et al. (2010) suggests that *F. selysi* ant workers originating from monogyne colonies may be able to mount a comparatively higher immune response for the same level of pathogen exposure than workers originating from polygyne colonies, simply because of their larger body size. Karl et al. (2011) investigated the effects of temperature extremes on fitness-related adult traits of the tropical butterfly *Bicyclus anynana* and concluded that there seems to be competing energetic demands between the immune system and general metabolism. When diapause temperature was high, larger queens had significantly more resources left for their immune functions than the smaller ones, possibly because their fat resources lasted longer and were large enough to cover for their basic metabolic needs over the whole diapause period. Smaller queens in the same treatment group, however, were not able to allocate as much energy to immune functions (hemolymph protein concentration and PO activity). The size-related differences I found in this study might be a consequence of the lower mass-specific metabolic rates of larger queens, and the ability of larger queens to allocate more resources to immune functions without compromising their other metabolic needs during diapause.

3.3.2 Sex ratio variation in *B. lucorum*

My fourth aim was to study the effect of queen size and worker population on the sex ratio and colony characteristics of the native white-tailed bumblebee *B. lucorum*. The fresh weight of the queens at the start of nest founding varied between 292.0 mg and 912.2 mg, and the average weight was approximately 510 mg. The time for the queen to start laying eggs varied from five to 56 days and this is called egg-laying delay. Maturation time, from the laying of first eggs until the hatching of the first sexual offspring, varied between 27 and 121 days. The size of the worker population at the time when sexual offspring hatched varied between zero to 171. The colonies produced zero to eight gynes and one to 62 drones. The proportion of gynes in sexual offspring varied from zero to 0.78.

The egg-laying delay decreased with an increased weight of queen, i.e., heavier queens started to lay eggs earlier. Colony maturation time and the size of worker population at the time of the hatching of the sexual offspring increased with an increased weight of

queen. The size of worker population at the time of the hatching of the sexual offspring increased with an increased length of colony maturation time. The start weight of the queen had no straight effect on the proportion and number of gynes in the sexual offspring. However, the increase in the size of worker population at the time of hatching of sexual offspring, which increases with increasing weight of the queen, decreased the proportion of gynes in the sexual offspring. In addition, the increase in the size of worker population increased the number of males produced, which decreased the number of gynes produced, i.e., the large size of queen indirectly led to a lower number of produced gynes.

I found that as expected, queen size varies significantly and could act as one explanation for the male bias often observed in bumblebee populations. There are multiple suggestions why bumblebee colonies are seemingly male-biased (reviewed in Bourke 1997), but queen size and factors influencing the size of worker population have not been addressed before. Worker reproduction could at least partially explain the male-bias we detected in the colonies of larger queens. However, if the probability of survival and successful nest founding after hibernation is greater for larger queens because of their higher immune competence (Vesterlund et al. 2014), the population sex ratio of *B. lucorum* colonies would become increasingly male-biased after warm winters, and also lead to a decrease in the total number of new gynes. Further, a proportionally high number of late-switching, large colonies would eventually lead to a decrease in the size of the population. Thus, by decreasing their effective population sizes, global warming causing mild winter temperatures in boreal areas could potentially make bumblebee populations vulnerable to local extinctions.

3.3.3 Nectar Unavailability in the Spring

My fifth aim (paper V) was to determine how high spring temperatures affect the survival of bumblebee queens during food scarcity in the spring, and if phoretic mites have any effect on the queens during that time. Bumblebee queen survival after emergence from hibernation is very dependent on the availability of nectar and temperature, both of which can affect the risk of starvation. Some insects are very tolerant to starvation, but insects with high metabolic rates and rapid utilization of nutrient stores have a constant demand for energy. At a temperature of 20°C, the honeybees will starve to death after only a few hours without feeding (Waters & Harrison 2012). Starved bumblebee queens survived for a shorter time at 24°C than at 15°C. I expected this result because body functions are faster and the overall energy expenditure is higher during warm than during cold conditions. In a recent experimental study of ant ecology, temperature differences affect survival, and the use of energy reserves in social insects. Boreal wood ants (*Formica aquilonia*) use

their body fat resources faster at high than low temperatures, and they survive better when the initial fat body resources are high (Sorvari et al. 2011). *Bombus lucorum* queen size, however, did not affect survival time at either temperature in my study.

All starved queens survived approximately 8 days longer when they were kept at 15°C than at 24 °C. This means that if the queens have trouble finding food after emergence (which in *B. lucorum* is quite consistent with the daily mean temperatures of 14 to 15°C) queens have a better chance of survival if the temperatures stay low until flowers become more abundant. High spring temperatures, on the other hand, would starve the queens very fast unless they were able to continuously visit flowers. Low night time temperatures are likely to prolong queen survival in nature, although rapid post-hibernation temperature changes from high to low might also be quite stressful to the emerged queens in a low resource environment. This is because the queens have to balance between getting enough nectar to survive and to be able to fly during a warmer than average day, and on the other hand, having an adequate energy storage for overcoming the cold night.

Parasitellus fucorum mites existed on 65 percent (50 out of 77) of the queens, but they did not have an effect on survival under laboratory conditions, neither was there any connection between queen size and the number of mites on the individuals. The highest numbers (70-200 individuals) accounted on queens did not seem to indicate a poor body condition as we proposed, and it may be that only numbers high enough to make flying or moving difficult, would have a significant negative impact on the queens. The potential mismatch between insects and plants due to climate change may also depend on their different responses to, for example, chilling, moisture availability, competition, predation and extreme weather events that could affect phenology and also modulate the effects of cumulative heat units or photoperiod (Forrest &Thomson 2011). However, frost damage that results in a reduction in the number of flowers may pose a more severe threat to bumblebee queens than phenological asynchrony (Inouye 2008). Although there is evidence of some bee species advancing their phenology by approximately ten days during the past 130 years, most plants seem to be following the same trend (Bartomeus et al. 2011). If mean temperatures continue to rise, there might still come a point when some species reach their phenotypic plasticity limits, and either their range will shift, or they could become locally extinct.

3.4. Limitations of the Study

In Finland, bumblebees have been studied mainly in the field, and the research has been concentrating on their diversity or pollination biology, for example flower preference and

visitation (e.g., Teräs 1985, Alanen et al. 2011). To my knowledge, this thesis contains the first experimental laboratory studies conducted in native Finnish bumblebees. Bumblebees have not been reared for scientific purposes before in Finland either and only one person is rearing commercial nests in non-scientific facilities. In addition, most of the essential rearing information from abroad is very well kept by commercial companies. This required learning and modifying the methods used for rearing other species, and developing the facilities and conditions suitable for the study species, *B. lucorum*. The developmental groundwork was important and highly interesting, but it was also time-consuming and set some limitations on what and how to study during this limited time span of the thesis work. I could not either perform genotype analyses of the reared *B. lucorum* males to look at how many of them were reproduced by workers.

In addition to time constrictions, there were limitations in how the performance of queen bumblebees could be studied. When the effects of overwintering temperature were assessed, it would have been interesting to know how much fat the queens usually have before they go into hibernation. In this case, however, sample size was relatively small already and the main focus of the experiment was to measure queen condition after the overwintering period. Thus, it was more important to have enough living queens at the end of the hibernation period, than to sacrifice some of them to prior-hibernation measurements. For the same reason, we could not study the effect of temperature on the actual survival of queens during a full-length diapause period. In future studies, more nests could be reared to acquire additional queens, or the rearing method further developed to produce queens more efficiently.

One of my aims in the beginning of the research was to analyze how the proximity of greenhouses affects the occurrence of an alien bumblebee species in Southern Finland. Two years trapping of bumblebees resulted, however, to catch only four individuals of that species. Thus, there were no means to assess that question during the time span of the research. It would have been interesting to try to unravel the origin of the *B. terrestris* individuals that were captured by comparing in detail their DNA sequences with several individuals from commercial nests. However, at the time of the study I did not have this option, and to fully assess this question, non-commercial *B. terrestris* specimens would also be needed from areas far away from commercial use.

4. CONCLUSIONS AND FUTURE PROSPECTS

Queens have a special role in the lifecycle of bumblebees. Their successful overwintering, longevity, immune competence, and ability to establish a nest in spring are highly important for bumblebee populations. In addition, factors influencing the structure of colonies are essential in evaluating population level impacts related to, for example, hibernation survival or spring performance of the queens. The effects of temperature on bumblebees was of interest because global warming, warm winters in particular, could potentially harm the overwintering and spring survival of queens. I found that queen size is an important factor that influences whether or not a queen is able to go into hibernation, how it succeeds during a mild winter, and even what kind of colonies it produces the next summer. The effects of climate change could lead to population declines by affecting the sex allocation of colonies. I detected two alternative strategies of colony development that differ between large and small queens, and this could be very important for the survival of bumblebee colonies in fluctuating climatic conditions, especially in the boreal area, where the impact of climate change is rapidly becoming more pronounced.

The commercially used and reared *B. terrestris* is thoroughly studied (for example, Beekman et al. 1998, 1999, 2000, 2008, Gosterit & Gurel 2008, Gurel & Gosterit 2009), and both its importance as a pollinator and the negative impacts on native pollinators are well known. However, it is as important to investigate the closely-related native species, which are most likely to be affected by these introduced commercial species and strains. This thesis work gives new insights into the identification, overwintering, sex allocation, and spring survival of wild bumblebees, and helps in monitoring the possible dispersal of commercial bumblebee strains and also limits the risk of the parasites and pathogens they carry from spreading into nature. A new PCR-RFLP method, which is very valuable due to is practical applications, was introduced for identifying a cryptic *Bombus s. str.* bumblebee group. With this method, it is possible to use a wider sample data in bumblebee monitoring, including specimens from various general insect traps, such as pit fall traps. In addition to morphologically damaged samples, old museum samples should also be more easily identified with our method, because only short DNA-fragments are needed.

The next logical step after studying the effects of temperature on the performance of queens would be to widen the scope and look at differences at the population level. Long term data collected from natural populations, combined with detailed environmental data, could reveal some trends that cannot be detected in laboratory studies. Monitoring

a larger area around major greenhouses that use commercial bumblebee pollination in southern Finland could give a better idea of the occurrence of *B. terrestris* in nature. This data should be compared with areas far from the impact of greenhouses. Sequencing should be used in order to reveal the origin of any *B. terrestris* individuals to find out if they are spreading naturally or from the greenhouses. While gathering the data, pathogens and parasites should also be investigated both from commercial and natural bumblebee colonies. This would be essential for the risk assessment of imported bumblebees. In addition, to be able to make concrete conservation decisions, the multiple effects of pesticides especially to queens and their reproductive ability should be studied in multiple species. There are numerous studies published recently that imply a causal link between the usage of pesticides and the survival and reproductive ability of bumblebees and bees. These questions need to be answered to form a more complete picture of the causes of bumblebee declines and to sustain healthy, diverse pollinator communities for our future.

Bumblebees are a very essential group of pollinating insects, and their overall survival and diversity in nature could determine how we cope with problems related to climate change. Disturbances in the global pollination of crops and wild flowering plants have a great importance on ecosystem functions and the well-being of humans. Especially drought and heat-waves are a growing threat to our food plants and the pollinator communities needed to maintain them. We need more information about how different pollinator populations are going to be affected by changes in climatic conditions, and what implications are further to be expected in the plant communities dependent on insect pollination. Commercial pollination can be both helpful and destructive, and it is highly important to use it in a sustainable manner, minimizing the risks and harnessing the potential. Wise political decisions are needed for addressing questions such as what commercial species and strains to use, when it is better to prohibit the import altogether, how the monitoring and risk assessment should be done, and how pollinator diversity could be enhanced. Combining research with political conservation decisions will give us a better chance in managing climate change induced problems.

5. ACKNOWLEDGEMENTS

I would like to thank my main supervisor, Assistant Professor Jouni Sorvari for introducing me to the wonderful world of social insects. Furthermore, he took the initial risk to let me concentrate on bumblebees, although neither of us were familiar with them at the beginning. This opened up a whole new world to me, and after working with bumblebees for several years I realized that I am one of the very few people in this country that have expertise in bumblebee ecology. Although starting from scratch required a lot of work and was not always easy, it was worth every step, even the very last ones climbing uphill.

I would also like to thank my co-supervisor Dr. Anti Vasemägi for introducing me to Molecular Ecology. I spent countless hours at the genetics lab with my bumblebee samples trying to figure out how to make everything work. It must have been a challenge to explain everything for someone with just the basic knowledge of genetics and almost no lab experience. All those hours in the lab taught me, among other things, patience, problem-solving, communication skills, and the fine art of delegating.

During the thesis work I collaborated with several people and I would especially like to thank Dr. Thomas Lilley and Dr. Tapio van Ooik for their contribution to the overwintering experiment and the following publication. Senior Adviser Harry Helmisaari (SYKE) provided me with valuable information and views on commercial bumblebees and was always very interested in the new developments of my research. I desperately needed to learn how to manage the bumblebees: thank you very much Erkki Kaarnama for teaching me all you know about bumblebee rearing. Many thanks go to Professor Kari Saikkonen and Dr. Marjo Helander, who both have been an uplifting part of my academic career since the beginning. For support in the last climb I would like to thank Professor Pekka Niemelä and Assistant Professor Jon E. Brommer. I was also very lucky to get a devoted and efficient language checking by Aura Professional English Consulting, Ltd.

The Ecology section of the University of Turku has provided me the assistance needed for studying and the facilities for my research, and the staff has become an important part of my life, like a standard that I find very hard to part from. Thank you all for being there. Also the staff of Teglab in the Genetics section helped me many times in multiple ways and with them I was always able to come up with solutions to whatever problem I had managed to create.

I owe huge thanks to the lovely and lively people at the Animal Museum of Turku University, my regular morning coffee and lunch company. On top of sharing your knowledge about whatever question I was pondering with, you folks have given me

support and understanding during some tough times into my life. I feel privileged that I was welcomed into your team.

All of you PhD students that have at some point been part of my life at the University of Turku have also been invaluable for giving support. I will not go through everybody one by one, there are so many of you, and those who have been there know it already. I feel I have been at the University for ages, and many that were there when I started have long ago continued on – thank you all.

Special thanks go to the members of the CBEES-group; Riikka Elo, Salla Härkönen, Maria Kakko, and Marja Haatanen. Being part of the group studying social insects and their associates, they automatically shared the same interests, as well as frustrations, than I did during this project. Maria had an important role as the primary caretaker of my bumblebee colonies in the lab, and together we learned a lot about the secret lives of queens and their colonies. I would especially like to acknowledge my closest office-mate Marja. She was always there whenever I happened to need help with something. She has been an excellent listener, and I admire her work moral and punctuality. Most importantly, she has become a valuable friend, the kind that is very hard to find the older you get.

I don't think I could have done this without the endless interest, energy, and love from my children, Essi and Lauri. My father gave me all the right reasons to study, and my mother, grandmother and closest relatives provided me with the important non-academic viewpoints on my research.

Last but not least – thank you my dear husband Robert for saving me.

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